IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant : Nishino, N., et al.
Serial No. : 09/945,237
Filed : August 31, 2001
Title : NOVEL CYCLIC TETRAPEPTIDE DERIVATIVES AND PHARMACEUTICAL USES THEREOF

Art Unit : Unknown
Examiner : Unknown

Commissioner for Patents
Washington, D.C. 20231

PRELIMINARY AMENDMENT

Prior to examination, please amend the application as follows:

In the specification:

Replace the paragraph beginning at page 9, line 6 with the following rewritten paragraph:

-- In one aspect of the invention, among the above four amino acids, D-configuration may be chosen for the cyclic amino acid represented by general formula (IV), while the remaining three take L-configuration; or D-configuration may be chosen for the amino acids represented by general formulae (II) and (IV), while the remaining two take L-configuration. It should also be noted that in the cyclic peptide of interest, hydroxamic acid derived from the side chain carboxyl group in the amino acid of general formulae (V) is a site close to the enzymatically active site of histone deacetylase instead of the side chain of N-acetylated lysine, so in one aspect L-configuration is selected for the amino acid of general formulae (V) as in the case of naturally occurring lysine.--

CERTIFICATE OF MAILING BY FIRST CLASS MAIL

I hereby certify under 37 CFR §1.8(a) that this correspondence is being deposited with the United States Postal Service as first class mail with sufficient postage on the date indicated below and is addressed to the Commissioner for Patents, Washington, D.C. 20231.

February 11, 2002
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Lucille M. Begalla
Signature

Lucille M. Begalla
Typed or Printed Name of Person Signing Certificate

Replace the paragraph beginning at page 12, line 12 with the following rewritten paragarph:

--One-tenth of the amount to be used of the peptide is dissolved in DMF and adjusted to a concentration of 0.1 mM. To the DMF solution under ice cooling, a tertiary amine, e.g., diisopropylethylamine and HATU (0-(7-azabenzotriazol-1-yl)-1,1,3,3-tetramethyluronium hexaflurophosphate) is added, and stirred at room temperature for 30 minutes. Subsequently, 1/10 of the amount to be used of the peptide, diisopropylethylamine and HATU are added to the above DMF solution and stirred at room temperature for 30 minutes. These procedures were repeated 10 times in total to effect a cyclization reaction. After the reaction, the product (cyclic peptide) is extracted into ethyl acetate and then purified by flash chromatography using a silica gel column.--

B2

Replace the paragraph beginning at page 13, line 8 with the following rewritten paragraph:

B3

--In adition to the above synthesis methods, the above compounds may also be synthesized by methods utilizing solid phase synthesis.--

Replace the paragraph beginning at page 13, line 11 with the following rewritten paragraph:

--A pharmaceutically acceptable salt of the cyclic tetrapeptide derivative according to the present invention means, for example, a salt with a pharmaceutically acceptable inorganic acid, such as hydrochloride, and a salt with a pharmaceutically acceptable organic acid, such as acetate, if the derivative has basic nitrogen atoms.--

B4

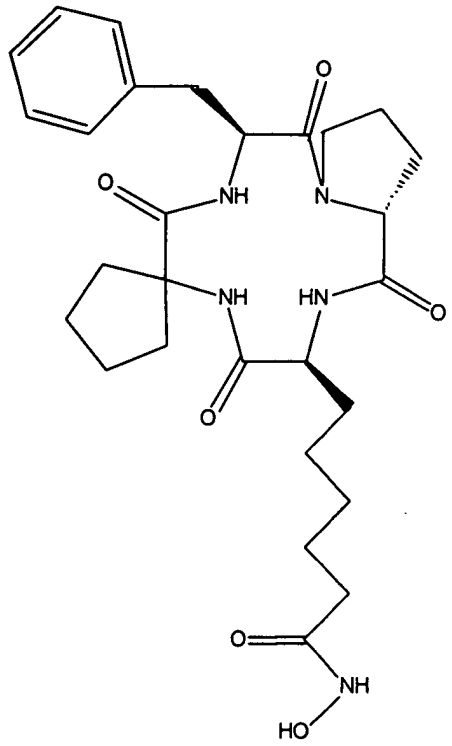
Replace the paragraph beginning at page 20, line 16 with the following rewritten paragaph:

--In the following examples, abbreviations for non-naturally occurring amino acids mean the following amino acid residues:

Aib:	2-aminoisobutyric acid:
Asu(NHOH):	2-amino-7-N-hydroxycarbamoylheptanoic acid;
Acc5:	1-aminocyclopentane-1-carboxylic acid;
Acc6:	1-aminocyclohexane-1-carboxylic acid;
Acc7:	1-amioncycloheptane-1-carboxylic acid;
Acc8:	1-aminocyclooctane-1-carboxylic acid;
1Ain:	1-aminoindane-1-carboxylic acid;
2Ain:	2-aminoindane-2-carboxylic acid;
Pip:	pipecolic acid;
Cha:	cyclohexylalanine.--

Replace the paragraph beginning at page 24, line 11 with the following rewritten paragaph:

--Example 1: Synthesis of CHAP-54; cyclo(-L-Asu(NHOH)-Acc5-L-Phe-D-Pro-)



B6

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Replace the paragraph beginning at page 36, line 9 with the following rewritten paragaph:

B7 Ins C1 --Step 7: Cyclo(-L-Asu(OH)-D-Cha-L-Ile-D-Pip-) and cyclo(-L-Asu(OH)-D-Cha-L-Ile-L-Pip-)--

Replace the paragraph beginning at page 36, line 26 with the following rewritten paragaph:

B8 --Step 8: Cyclo(-L-Asu(NHOH)-D-Cha-L-Ile-D-Pip-) and cyclo (-L-Asu(NHOH)-D-Cha-L-Ile-L-Pip-)--

Replace the paragraph beginning at page 40, line 15 with the following rewritten paragaph:

--Said B16/BL6 cells were inoculated on a 96-well microplate at a cell density of 5000 cells per well, each well containing 200µl of said medium. After culturing for 24 hours, 10µl of a sample containing a given amount of the stock solution of a test compound which had been diluted in the medium was added and cultured for additional 72 hours. Thereafter, each well was washed once with PBS (phosphate buffered saline) and floating cells and the medium were removed. Then, the well was treated with 0.1% glutaraldehyde solution for 3 minutes to fix the cells.--